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MODELLING THE CONTROL OF CELL PROLIFERATION BY AN ANTI-CANCER AGENT

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Abstract

A mathematical model has been developed which describes human cell growth in the presence of topotecan (TPT), an anti-cancer agent. Previously, a model was developed for the pharmacokinetics (PK) of TPT; this provided a description of the activity of TPT and the subsequent delivery of the active form to the nuclear DNA target. This model is described briefly in this paper. The development of a new pharmacodynamic (PD) model for cell growth, based on a well-known cell cycle model is then discussed. The linking of the PK model to the PD model is considered with respect to the modulation of cell growth. Our PK – PD model has good agreement with *in vitro* experimental data. In a clinical context, it is intended that the model will be used for discovering routes for drug resistance and eventually as a tool for improving drug treatments for patients.

1. Introduction

The development of anti-cancer agents is an expensive and lengthy process. The aim is to find and develop agents for drugs which possess one or more therapeutic, toxicological and pharmacokinetic properties. These are initially assessed through laboratory-based investigations conducted *in vitro* to establish proof-of-principle and subsequently *in vivo* analyses where the pharmacokinetic (PK) and pharmacodynamic (PD) properties are assessed. During this stage, agents are either rejected or developed further through to clinical trials.

The use of mathematical models which utilise ideas from systems engineering to aid this process is now firmly established. This is a consequence of ever-increasing computing power and, more importantly, recent developments in measurement technologies which have enabled laboratory experiments to measure accurately the concentrations of the agent and other active metabolites. Mathematical models that have been developed independently of biological experiments are often too complex and lack validation for any real practical purpose.

In the present paper, the development of a mathematical model which describes the *in vitro* kinetics of the anti-cancer agent topotecan (TPT) on human osteosarcoma cells is described. Clinical evidence for the cytotoxic properties of topotecan (TPT) against lung, breast and ovarian cancers is well known [1]. TPT is a water-soluble derivative of camptothecin, which is an alkaloid isolated from a Chinese tree, *Camptotheca acuminata*. TPT acts as an inhibitor of topoisomerase-I [2], which is a nuclear enzyme involved in DNA replication and transcription. The enzyme unwinds supercoiled double-stranded DNA (dsDNA) by temporarily binding to and cleaving the strands, forming a cleavable complex. This process is tightly controlled and barely detectable. The cleavage is then coupled with religation to check and then restore the strands to form the original DNA complex. In the presence of TPT, the cleaved DNA-topoisomerase-I complex is stabilised, effectively inhibiting religation. As DNA replication continues the TPT-induced double-strand breaks that have formed prevent any further cell growth.

Part of the overall model was developed earlier to describe the pharmacokinetics (PK) of TPT [3]; this provides a description of the activity of TPT and the subsequent delivery of the active form to the nuclear DNA target. In the

present paper, the linking of this part of the model into another part describing the growth of cells is discussed. This new component, the pharmacodynamic (PD) part of the overall model, is based on the cell cycle model of Tyson and Novak [4]. The objective of the overall model is to show the dynamic interaction of the drug with its molecular target, and its impact on cell growth.

The model described in the present paper has been validated with specific live-cell experimental data from the Wales College of Medicine, where quantitative laboratory assays have been developed that enable single cell events to be tracked and measured with time [5]. These data are used to validate and search for cellular routes of the evasion of the drug, i.e. drug resistance. Ultimately, the aim of the model is to design and predict the consequences of tailored drug treatments for individuals. With this in mind, care has been taken to develop a highly flexible model, which is capable of predicting cell response pathways for use in drug discovery.

The layout of the remainder of this paper is as follows: Section 2 introduces the PK model, while Section 3 introduces the PD model; Section 4 discusses the linking of the two models, and Section 5 describes how well the PK-PD model predicts the experimental behaviour.

2. The Pharmacokinetic (PK) Model

The PK model [3], which is shown in Figure 1, is compartmental, with three regions:

- The MEDIUM, into which TPT is added;
- The EXTRACELLULAR region, from which TPT can enter the cells;
- The CELLULAR region, which is further divided into the CYTOPLASM and the NUCLEUS.

The model describes the chemical reactions that occur when the drug moves from the medium to the cellular regions and the nucleus. Under physiological conditions TPT is able to change its chemical structure, from an active (closed-ring, lactone) form (TPT-L) to an inactive (open-ring, hydroxyl acid) form (TPT-H); this is a reversible reaction. Both TPT-L and TPT-H exist in the medium, the extracellular region and the cytoplasm, but it is assumed that only TPT-L exists in the nucleus [3].

The model is described by the following system of Ordinary Differential Equations:-

$$\frac{\mathrm{d}L_m}{\mathrm{d}t} = -\left(k_{om} + k_{mi}\right)L_m + k_{cm}H_m + k_{mo}v_0 L_e \tag{1}$$

$$\frac{\mathrm{d}H_m}{\mathrm{d}t} = k_{om}L_m - \left(k_{cm} + k_{mi}\right)H_m + k_{mo}v_0 H_e \tag{2}$$

$$\frac{\mathrm{d}L_e}{\mathrm{d}t} = \frac{k_{mi}}{v_0}L_m - \left(k_{mo} + k_{om} + k_i\right)L_e + k_{cm}H_e + \frac{k_e}{v_1}L_c \tag{3}$$

$$\frac{\mathrm{d}H_e}{\mathrm{d}t} = \frac{k_{mi}}{v_0}H_m + k_{om}L_e - \left(k_{cm} + k_{mo}\right)H_e \tag{4}$$

$$\frac{\mathrm{d}L_c}{\mathrm{d}t} = k_i v_1 L_e - \left(k_e + k_{oc}\right)L_c + k_{cc}H_c + k_{dl}v_2 L_n - k_b\left(B_T - L_n\right)L_c \tag{5}$$

$$\frac{\mathrm{d}H_c}{\mathrm{d}t} = k_{oc}L_c - k_{cc}H_c + k_{dh}v_2 L_n \tag{6}$$

$$\frac{\mathrm{d}L_n}{\mathrm{d}t} = \frac{k_b}{v_2}\left(B_T - L_n\right)L_c - \left(k_{dl} + k_{dh}\right)L_n \quad . \tag{7}$$

The subscripts *m, e, c* and *n*, denote the medium, extracellular region, cytoplasm and nucleus, respectively, with the volumes of the compartments denoting these regions being V_m, V_e, V_c and V_n. In the equations, v_0, v_1 and v_2 are volume ratios given by $v_0 = V_e/V_m$, $v_1 = V_e/V_c$ and $v_2 = V_n/V_c$. As noted above, it is assumed that all drug in the nucleus is bound and that only TPT-L binds to DNA. In all other locations reversible hydrolysis occurs and is modelled as a two-compartment sub-model; for example, the rate constants k_{om} and k_{cm} define the ring opening (for TPT-L) and ring closing (for TPT-H) respectively in the medium. The mixing between the medium and extracellular region is modelled by first-order flows, with k_{mi} being the rate constant for the flow into the extracellular region from the medium and k_{mo} being the rate constant for the flow in the opposite direction. The flow across the plasma membrane between the extracellular region and the cytoplasm is assumed to be for TPT-L only [6], and is again modelled by first-order flows.

The rate at which TPT-L binds to DNA is assumed to be proportional to the product of the concentration of TPT-L in the cytoplasm, $L_c(t)$, and the free concentration of binding sites $B_F(t)$ (in accordance with the Law of Mass Action). The concentration of free sites is given by: $B_F(t) = B_T - L_n(t)$, where L_n is the concentration of bound drug. Dissociation of bound drug is assumed to be first order,

yielding either cytosolic TPT-L (with rate constant k_{dl}) or TPT-H (with rate constant k_{dh}).

The drug is injected as a bolus of dose, D, at $t = 0$, yielding the initial conditions: $H_m(0) = L_e(0) = H_e(0) = L_c(0) = H_c(0) = L_n(0) = 0$; and $L_m(0) = (1+v_0)D$. The non-zero initial condition accounts for the injection of drug into the medium before mixing with the extracellular region takes place. Experimental measurements were of the concentrations of TPT-L and TPT-H (combined) in the extracellular region and the cytoplasm and of TPT-L in the nucleus. With this input and these measurements, it was found that the model parameters were globally (uniquely) structurally identifiable [3]. The model parameters were then estimated from the data using the software package FACSIMILE [7], with good visual fits to the data and low residual sum of squared error obtained for almost all of the model parameters.

3. The Pharmacodynamic (PD) Model

The pharmacodynamic (PD) model is based on the cell cycle model of Tyson and Novak [4], shown in Figure 2. The cell cycle comprises four phases (G1, S, G2, and M) where a single cell's cellular machinery and chromosomes are replicated in order to undergo division [8]. In the first 'gap' or G1 phase, the chromosomes are unreplicated and the cell is prevented from entering cell division. During transition from this phase to the Synthesis or S phase the cell receives a signal to commit to replication of the genetic material. As new DNA is synthesised chromatids are duplicated in the S phase. In the G2 phase, a cell prepares for commitment to mitosis (M). In M, the nuclear material condenses, the nuclear membrane is lost, spindle fibres pull chromatids apart and a cleavage furrow forms, separating the two new daughters. The newly formed daughter cells regain their nuclear membranes and de-condense the nuclear material, establishing a new G1 state. These processes are distinct and tightly regulated temporally and demand the spatial separation of the controlling molecules [9].

The cell cycle may be thought of as two alternative states, with G1 and S-G2-M separated by Start and Finish transitions [4]. Start is defined as the time where cell replication begins and Finish where DNA replication is complete. If there have been any problems, for example with DNA replication or chromosome alignment, the cell

cannot divide as the Finish transition cannot begin. With this in mind, TPT is believed to be S phase specific as the cleavable complexes (representing the trapped drug-DNA topoisomerase complex) lead to dsDNA breaks by collision with actively replicating DNA. This prevents further cell cycle progression. It has been shown that many cells resist the effect of topotecan by failing to enter the S phase or replicate DNA during exposure [10]. By blocking entry to the S phase, it is possible to halt the growth of cells.

The cell cycle model of Figure 2 is regulated by the interaction of Cyclin dependent kinases (Cdk's) and Cyclin B with a group of proteins called the Anaphase Promoting Complex (APC). Cdk's induce downstream processes by activating selected proteins, and the APC marks specific components to bring the cell cycle to an end. The modified cell cycle model, which is the PD model used in this paper, is shown in Figure 3. The heart of the model is the relationship between Cdk/Cyclin B and APC. The principal model equations are:

$$\frac{\mathrm{d}[APC]}{\mathrm{d}t} = \frac{\left(k_3' + k_3'' A\right)\left(1-[APC]\right)}{J_3 + 1 - [APC]} - \frac{k_4 a [CdkCycB][APC]}{J_4 + [APC]} \tag{8}$$

$$\frac{\mathrm{d}[CdkCycB]}{\mathrm{d}t} = -k_1 - \left(k_2' + k_2''[APC]\right)[CdkCycB] \tag{9}$$

$$\frac{\mathrm{d}a}{\mathrm{d}t} = ga\left(1 - \frac{a}{a_*}\right) \tag{10}$$

where the components in square brackets are concentrations; the k_i's are rate constants; J_3 and J_4 are Michaelis-Menten constants; a is the size of the cell; a_* is the maximum size of the cell; and g is the specific growth rate.

4. The PK-PD Model

As noted in Section 1, quantitative laboratory assays have been developed at the Wales College of Medicine that enable single cell events to be tracked and measured with time [5]. The cells in this application were human osteosarcoma cells, which had been engineered to carry a fluorescent reporter (an enhanced form of Green Fluorescent Protein eGFP) fused to cyclin B1, one of the master regulators of cell cycle progression. The expectation was that the cyclin signature would show a

ramping up as cell progresses through the cell cycle and then betray their arrest or commitment to cell division. The cells were treated with 0.01, 0.1, 1 or 10 μM of TPT for 1 hour at 37°C and then washed out. Cultures were then incubated at 37°C over 48 hours, and during this time, they were tracked using a time-lapse digital camera to measure GFP fluorescence at high resolution every 20 minutes. A mitotic event gave a large peak in the fluorescence time record, and it was found that the time between such events increased as TPT was added, from around 20 hours without TPT to around 36 hours with 10 μM of TPT. This increase suggested coupling the PK model for TPT with the PD (cell cycle) model. Coupling a PK model with a PD model in this way is a relatively new idea [11]. A recent example [12] examined the effect that a lack of extracellular oxygen (hypoxia) would have on the growth of normal and cancerous cells.

There is strong evidence [10] that the PK model should be coupled to the S-phase of the PD model, so giving the combined PK-PD model shown in Figure 4. The transducer box in this figure indicates that the active TPT causes DNA damage that signals stress and sends an arrest signal to the Cdk/Cyclin complex master regulator. This is considered to be an overall equilibrium – hence the forward and reverse arrows in the transducer box. The principal PD model equations (8) and (10) remain the same, but there is now an additional term in Equation (9) for the input from the PK model:

$$\frac{\mathrm{d}[CdkCycB]}{\mathrm{d}t} = -k_1 - \left(k_2' + k_2''[APC] + k_3''[L_n]\right)[CdkCycB] \quad . \tag{11}$$

5. Comparison of Model Predictions and Experimental Data

Figure 5 compares the measured data with the model predictions, in the absence of TPT (Figure 5A), and the presence of 1 μM and 10 μM of TPT (Figures 5B and 5C, respectively). The live cell data are denoted by the solid line and the model predictions by the dash-dot curve. The Start and Finish cell cycle transitions can be identified easily and have been marked on Figure 5A. Whilst the G2 phase is not clear, due to the low signal-to-noise ratio in the experimental data, it is possible to deduce the other phases. As the cell divides, indicated by the spike, the growth of either one of its daughter cells can be monitored.

The model predictions present a very good approximation to the experiment. (The accuracy of the fits to the experimental data have been determined, in the first instance by calculating the least squares fit. This resulted in r^2 values of between 0.91 and 0.95, which indicate a very good fit.) The main outcome is that the model is capable of accurately predicting the time-event characteristics. The model is also capable of predicting the Start and Finish transitions and the cell cycle phases. This is partly a result of the fact that the parameter values are constantly changing from phase to phase and cell to cell. It can be observed in Figures 5B and 5C that TPT extends the S phase and hence the overall time to mitosis.

6. Conclusions

This paper has described the development of a PK-PD model describing the behaviour of human osteosarcoma cells with time. The model is able to predict the effect that varying doses of topotecan have on cell growth, giving very good agreement with live-cell experimental data. The model provides a framework to investigate the role of the signals from damaged DNA (i.e. originating from active drug delivered to critical sites as described by a PK model) to induce the arrest of cell cycle traverse by engaging aspects of the cell cycle machine. It is this critical area that can be dysfunctional in a tumour cell to varying degrees and potentially accessible to modelling.

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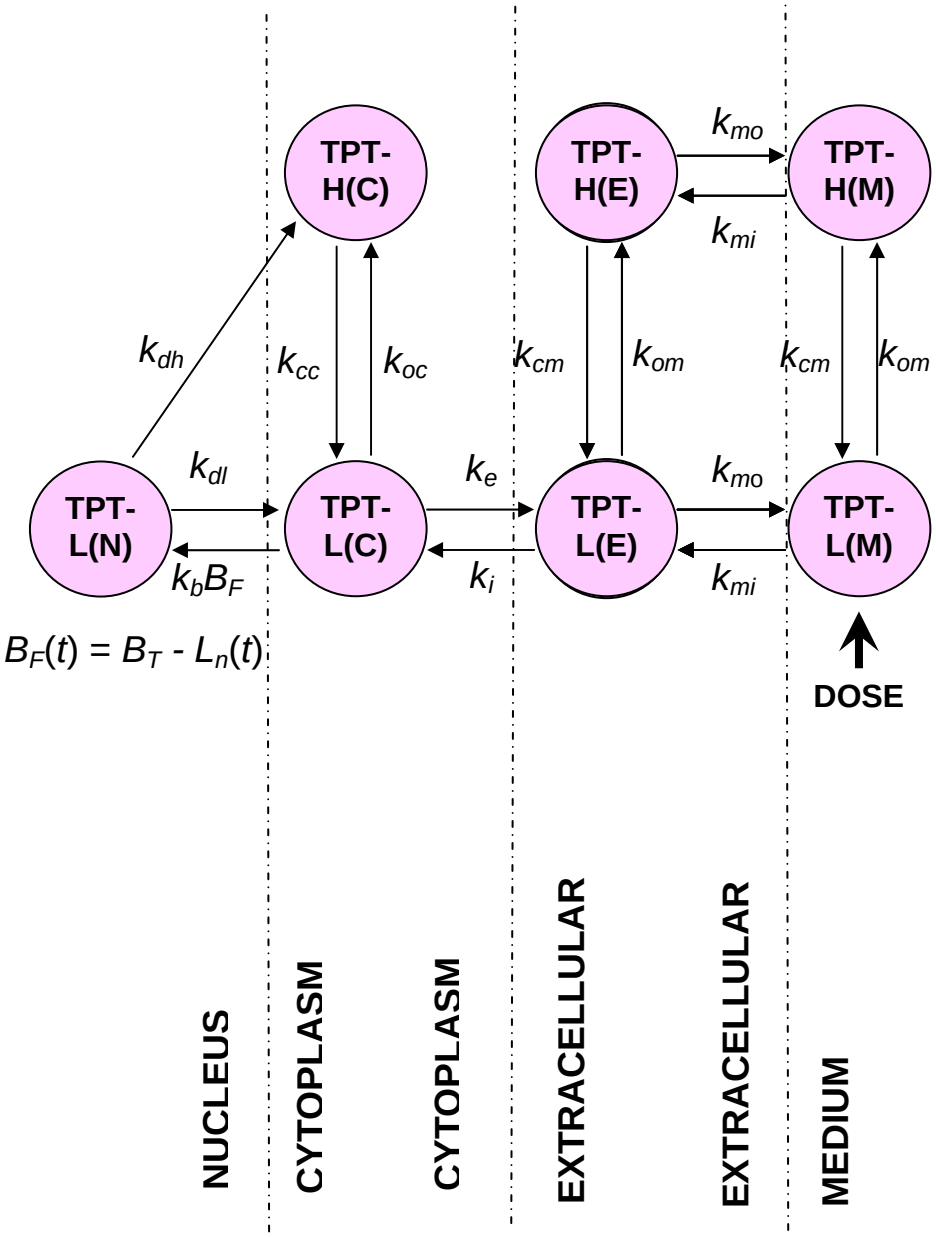


Figure 1: The Pharmacokinetic (PK) Model

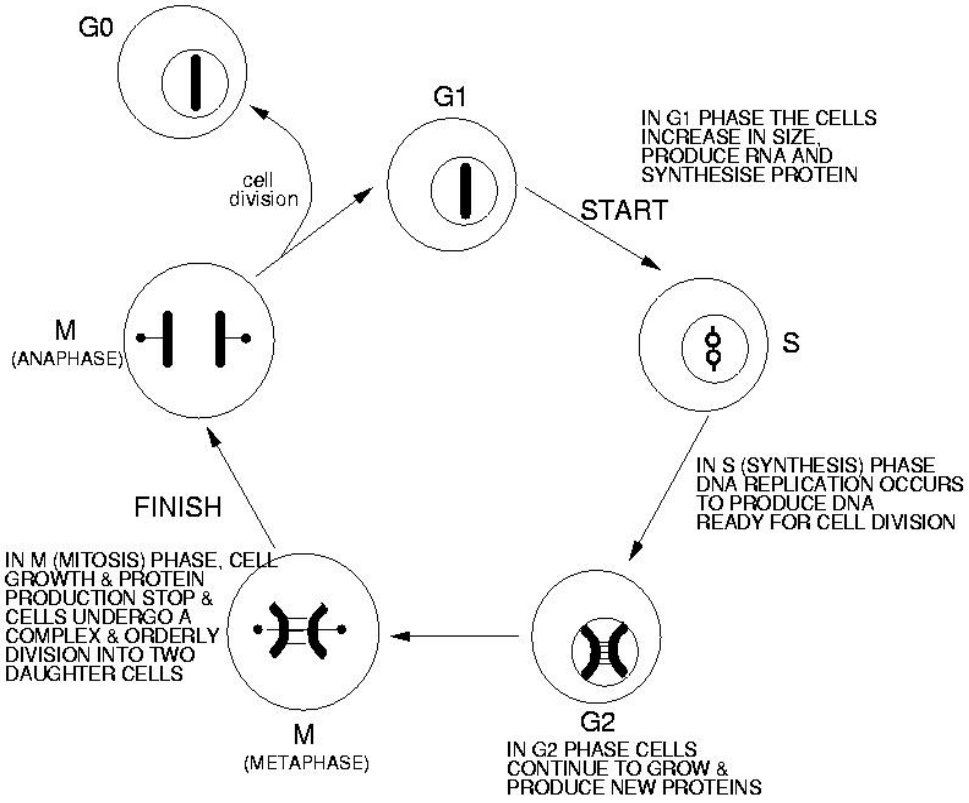


Figure 2: The cell cycle model, after Tyson and Novak [4]

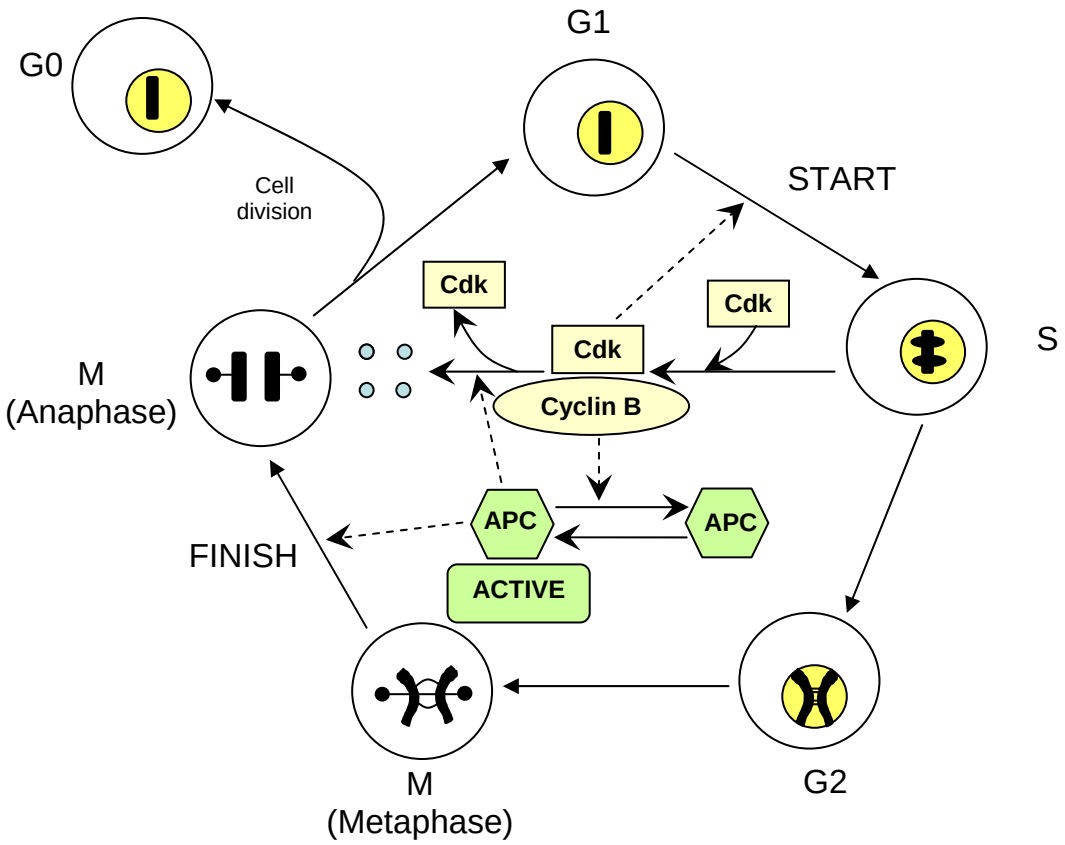


Figure 3: Modified Cell Cycle Model – the Pharmacodynamic (PD) Model

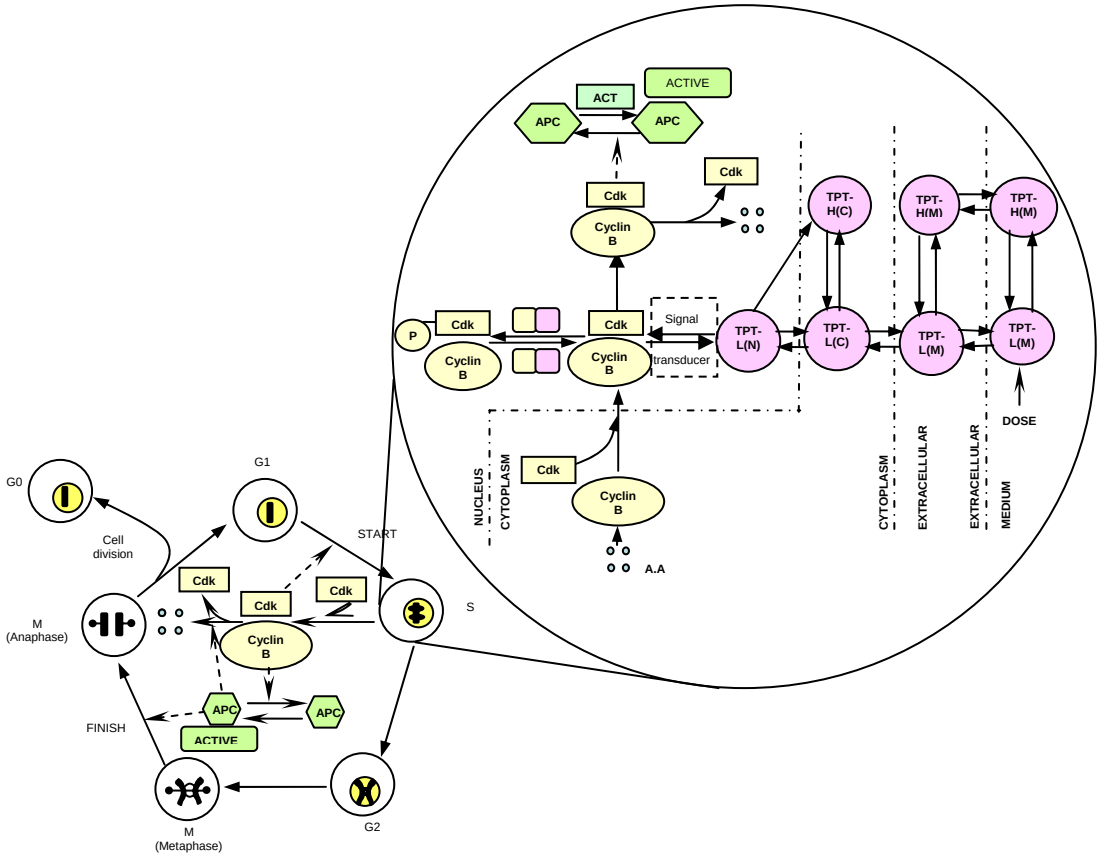
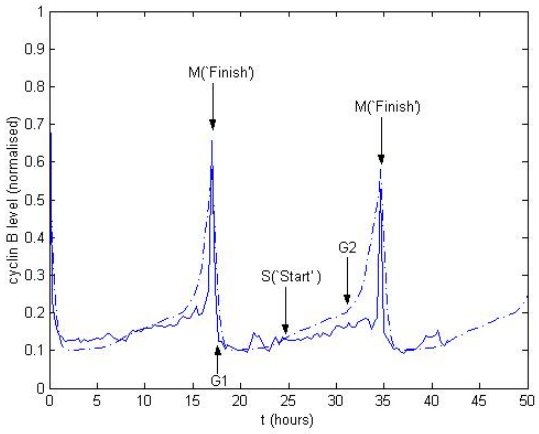
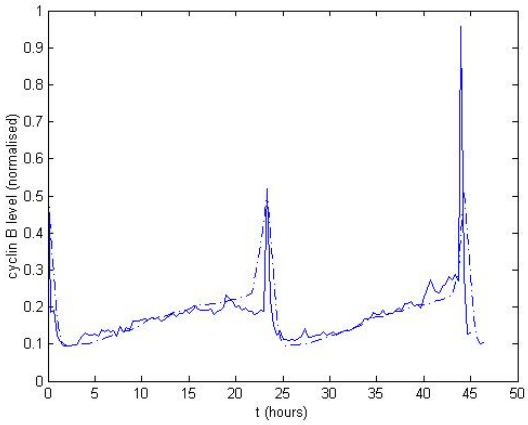


Figure 4: The combined PK-PD Model



(a)



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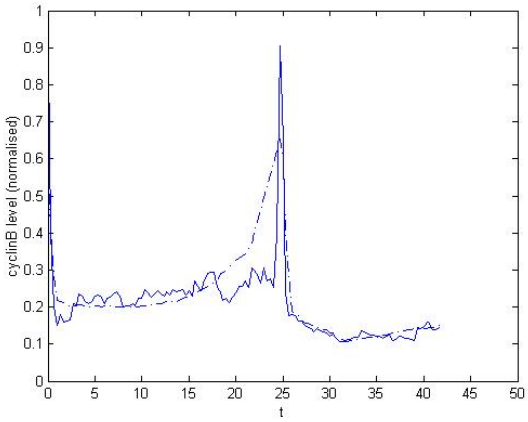

(c)

Figure 5: Variation of Cyclin B with time, (a) with no TPT, (b) with 1 μM of TPT and (c) with 10 μM of TPT. Solid line: experimental data; Dotted line: model prediction.